Transforming biosafety: a new philosophy in synthetic biology

Introduction

Context of report

As the field of synthetic biology gains momentum, slowly becoming integrated within molecular biology laboratories across the globe, the discussion about synthetic biosafety is becoming more and more important. The International Genetically Engineered Machine (iGEM) competition, created in 2003 at the Massacheusets Institute of Technology in Boston, which places the tools of synthetic biology into the hands of undergraduate scientists, has accelerated growth and activity within the field to an unprecedented degree. Biosafety regulations, however, have failed to match this growth, such that the current guidelines in many countries are essentially irrelevant to ensuring the safety and indeed responsibility of conducting certain synthetic biological research. This report, authored by the 2015 iGEM team from UNSW Australia, aims to explore the nature and ramifications of such disjunction. Of most importance, however, it suggests a shift within the philosophical paradigm of synthetic biology to better perceive the potential risks of genetic modification.

Project Endosynbio

Project Endosynbio is illustrative of the biosafety challenges facing synthetic biology. Involving the use of parts from RG1 to RG3 organisms, including virulence genes and toxins, in order to create invasive bacteria, this project raises significant red flags. It is clear, however, that the current regulatory bodies and guidelines are not equipped to manage these. The following report will thus be discussed in reference to the Endosynbio project.

Issues considered

This report canvases a range of issues regarding synthetic biology biosafety, beginning with the iGEM competition from conception to maturation. New philosophical perspectives are offered on transformations in synthetic biology. With reference to Project Endosynbio, we elucidate the pitfalls

of current perspectives regarding chassis and gene donor. Finally, a consideration of the current regulations for gene modification in Australia is provided.

Safety and security in the iGEM competition

Assessing biosafety and security is an important component of participating in the iGEM competition. All teams are required to 'check in' potentially hazardous parts or ideas, whilst some parts, from RG4 organisms, are not permitted for use. An official safety officer is appointed who maintains contact with each team as their projects progress. However, these measures may be insufficient to deal with the explosion of synbio in the global community.

As the iGEM competition has grown and matured, a range of measures have been gradually introduced 2003 to raise biosafety awareness. These measures were developed in recognition of the range of safety standards and enforcement across different teams and countries, rapid growth in participation and importantly, to encourage young, amateur scientists to think more critically about the potential risks of their work. A large part of this is the mandatory safety 'check-in's' for any 'red flagged' parts which alerts registry users to the risks of certain parts, whilst allowing competition administrators to keep track of any potentially dangerous ideas. Another key part of this is two non-negotiable rules: no teams are to work with RG3 or RG4 organisms, and no modified organisms or products are to be released into the environment. The remainder of the safety advice from consists of questions designed to stimulate thought and encourage safe design and risk management.

Despite these endeavours, some members of the scientific community are concerned about how the iGEM competition has accelerated the uptake rate of synthetic biology, to the detriment of safety. The iGEM movement has encouraged amateur biologists, engineers, designers, IT specialists and further to engage with synthetic biology in novel and creative ways that rarely place risk in the foreground (1). There is a significant risk in this diffusion of skills, especially regarding users without a grounding in biology. By accelerating access to gene technologies, skills and data, participants may not have time to develop the appropriate ways of thinking to handle the work safely. This lag is especially seen in the context of the iGEM competition, wherein young teams entering an exciting period of their lives may sidestep the broad issues in biosafety.

There also appears to be intrinsic safety flaws in the Registry Design. Despite the administrative ‘red flag’ system, dangerous parts are still freely accessible to competitors and other users and may be handled inappropriately. As an inaugural team in 2015, for example, BABS UNSW Australia was sent a live agar stab of bacteria constitutively expressing virulence factors from RG2 gene donors, a device which had been characterised to successfully invade eukaryotic cells in 48% of exposures. This situation indicates problems both with Registry administration, and a lack of experience on behalf of the competitors. Although Escherichia coli K12 is usually an innocuous bacteria, the virulent genes meant it had been transformed into a very different beast which was mishandled in a way that was potentially very dangerous, with unforeseen consequences. The ease of how this situation developed highlights the need for synthetic biosafety reform.

Whilst biosafety has improved significantly in the past twelve years, it is clear that fundamental shifts within iGEM and the wider synthetic biology community are necessary. It is necessary to return to first principles, and reconsider the way in which we think about design of our molecular biological experiments, paying particular regard to chassis and gene donor, in order to maximise safety.

A new philosophy: synthetic pseudostrains

What defines a 'new organism'?

Synthetic biology is based on the framework of a distinct 'chassis' organism being transformed with gene constructs, which are modular and often transient relationships. Transformation of plasmids is often semi-permanent, with research indicating that not only may plasmids leak from a transformed organism, but the important concept of plasmid incompatibility leading to loss of heterologous plasmids throughout generations, due to maintenance of a standard copy number. However, the fact remains that transformation is a one-way protocol in molecular biology (it is unlikely that any attempt to reverse the process would leave the bacteria viable). In the lab, fast-growing, stable lab bacterial strains can be rendered competent rapidly and cheaply; commercial competent cells are also available cheaply over the internet; these cells may be rapidly destroyed via autoclaving or chemical sterilisation. Essentially, there is no impetus for a scientist to reverse plasmid uptake. As such, once a cell has gained a plasmid, you cannot make the decision nor take the action to remove it. The thought becomes even more interesting when considering one bacteria of project Endosynbio, Synechocystis

PCC6803. In order to transform this bacterium, integrative plasmid vectors targeted to specific, non-coding regions of DNA are used to insert naked DNA directly into their 12 bacterial chromosomes. Rather than just carrying a new plasmid, this bacteria now has a novel genome.

When we use the term ‘chassis,’ however, defined as a ‘supporting framework,' 'skeleton' or 'shell,’ we ignore this permanence. By extension, we imply that the bacterium is merely a vector for gene expression, rather than considering its intrinsic characteristics and the interplay between organism and gene. The current philosophical paradigms of synthetic biology do not support the idea that each transformation is a new organism being created – however, we believe a shift in this philosophy would result in a shift in perspectives of biosafety.

Thus, we believe it is a valid position to consider the bacteria of project *Endosynbio*, and indeed any other transformed bacteria, as potentially transient, however also potentially permanent, 'pseudostrains', a term we define to mean:

New bacterial strains which have been created by synthetic genetic alterations, including genome modifications and transformation.

We believe that promoting this paradigm shift will help future iGEM teams and synthetic biologists to better assess the risks of their projects.

Case study: pathogenic pseudostrains in Endosynbio

Project Endosynbio involved the transformation of three bacterial chassis with the virulence factors of two RG2 bacterial pathogens to create invasive bacteria. This project is particularly illustrative of the importance of considering synthetic pseudostrains.

<u>Original organisms of the invasin/listeriolysin O invasion system</u>

The invasin/listeriolysin O system for bacterial invasion of a host eukaryote is not novel to *Endosynbio* and indeed, has been used by independent research labs and past iGEM teams alike (2,3). We believe that the current risk assessments and biosafety management of this system,

however, are lacking. Little is known about the nature of the hybrid invasive plasmid, as there is little characterisation and a limited range of tested chassis.

To firstly introduce the two gene donors:

Yersinia pseudotuberculosis is a risk group II pathogen responsible for a range of acute enteric diseases, which are mostly self-limiting however may progress to more severe forms of disease (4). Humans are believed to be incidental hosts, due to its rare occurrence and its infection mainly in wild mammals. The infectious dose is believed to be greater than 10^8 organisms, transmitted enterically either human-to-human or zoonotically. A key feature of its pathogenesis is the invasin gene, which induces endocytosis such that the bacterium is ingested and able to infect individual cells.

The second of our invasive genes, listeriolysin O, is sourced from *Listeria monocytogenes,* another rare human pathogen known for causing a severe condition known as listeriosis with up to a 50% mortality rate (5). It is especially infectious to the immunocompromised and pregnant women, with an infectious dose of as little at 0.1 million CFU as compared to 10 million for healthy patients, and transmitted enterically.

Role of the chassis

Considered in isolation, *Y. pseudotuberculosis* and *L. monocytogenes* are not incredibly dangerous. A range of intrinsic and extrinsic factors, such as their rare incidence, low transmission rate, host specificities and high infectious dose, have been considered to decide on their ranking as RG2 organisms. However, when considering the use of individual genes – a single virulence factor, for example – transformed into a chassis, even of a lower RG, there is no guarantee that this new pseudostrain is also RG2. It is crucial to consider how transformation may alter or enhance the virulence and risk of the original organism. Two of the chassis in project *Endosynbio*, *Escherichia coli K-12 subsp. Dh5-alpha* and *Lactococcus lactis subsp. lactis*, will be discussed in relation to this ambiguity.

Regeneration of pathogenicity: E. coli Dh5-alpha

Dh5-alpha cells are a derivative of the non-pathogenic K12 strain of Escherichia coli. The original K12 strain, isolated from a convalescent diphtheria patient in 1922, has served as a model laboratory bacterium for molecular biology for decades and given rise to a range of substrains adapted specifically for cloning, expression or otherwise (6). These bacterium have multiple defects to their cell wall, including the absence of fimbriae, altered glycocalyx and absent capsular antigens, which prevents their colonisation of the gut wall and causing of disease. A range of studies into the safety of K12 use have proved that these are innocuous, RG1 organisms – so reliably safe, that they are exempt from reporting to the Office of the Gene Technology Regulator (OGTR), the governmental regulatory unit for gene technologies in Australia.

In nature, E. coli exists largely as a non-pathogenic human enteral bacterium, accounting for approximately 1 in every 1000 bacteria in the human intestine. Horizontal gene transfer via conjugation and transduction has meant, however, that the genome is highly promiscuous and a range of virulent strains are also present. These are important causes of gastrointestinal disease, including dysentery, watery diarrhoea and ulceration of the bowel wall.

Pertinent to Endosynbio is the enteroinvasive strain of E. coli (EIEC), an intracellular pathogen of human enterocytes as indicated in Figure 1 (7). This strain employs invasins to invade the cell wall in conjunction with lysosome-degrading enzymes to enter the cytoplasm. This invasion mechanism is very similar to the invasin-listeriolysin system used in *Endosynbio* and may be cause for concern. By conferring intracellular invasive ability to E. coli, are we undermining the safety of the K12 strain and essentially working with a synthetic EIEC pseudostrain?

We believe the answer to this question is yes. Despite this, the OGTR would not consider this organism dangerous enough to acknowledge. This indicates a significant gap between the perspectives and regulation of genetic modification and the heart of the science. If we *do* consider this K12 transformant an EIEC pseudostrain, then a raft of new implications emerge. Suddenly, a new pathogen has been created, conceived with no data relating to its pathogenicity, hosts, transmission, environmental reservoirs or any other characteristic that we typically investigate to minimise the risk of natural microorganisms. Whilst it would be impossible to identify these characteristics in a synthetic pseudostrain, it is important to consider them when deciding how to manage your organism.

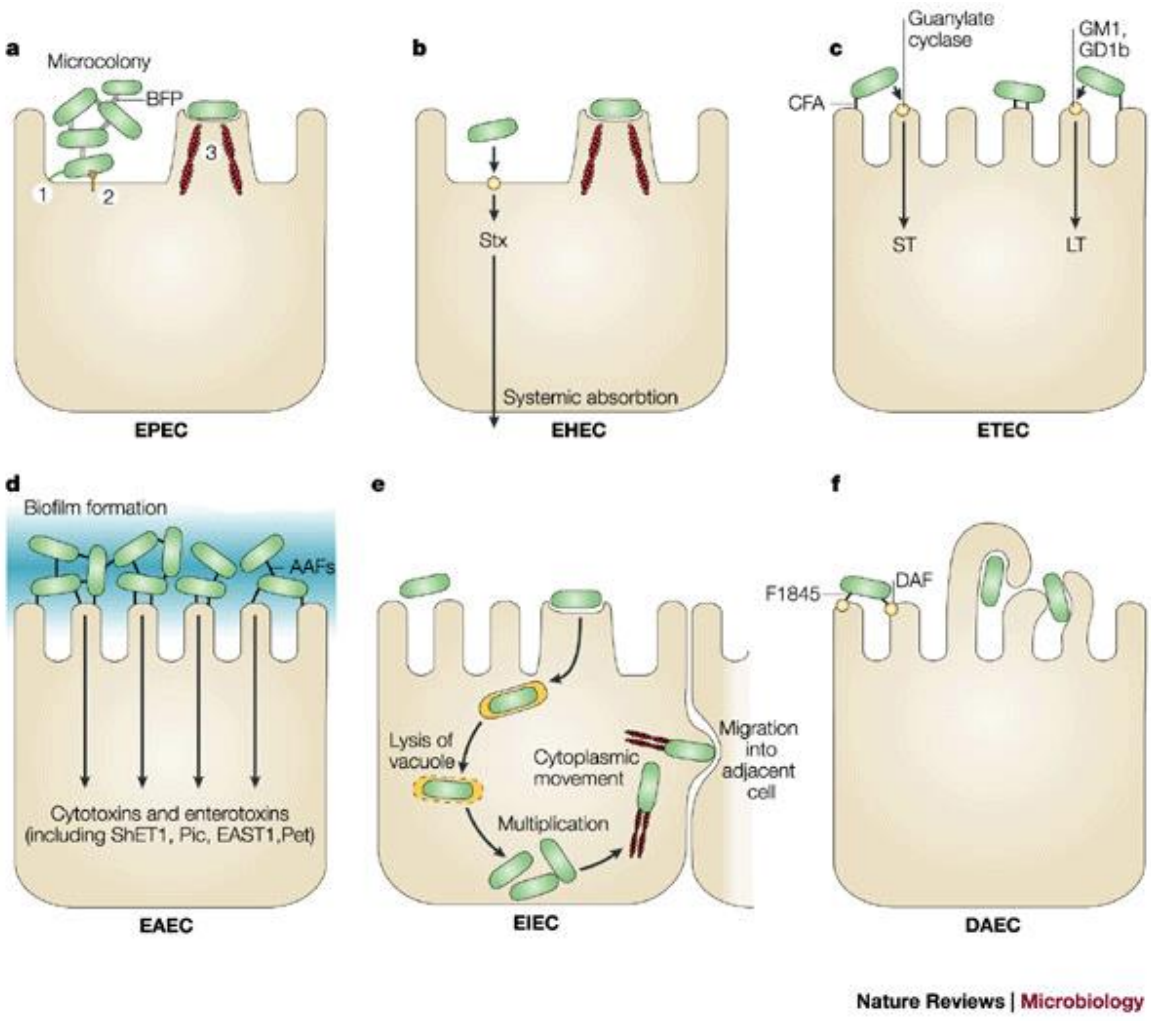

Figure 1. Pathogenic E. coli strains (7).

Regulation of gene technology in Australia

Risk assessments in Australia via the Office of the Gene Technology Regulator and the Gene Technology Act 2000

In Australia, work in synthetic and molecular biology involving genetic manipulation is monitored by the OGTR, which enforces the Gene Technology Act 2000 with the intention of reducing health, environmental and other risks. As has been briefly indicated thus far, however, the current Australian guidelines do not support the risks of work in synthetic biology by assessing integrated risk from donor and chassis and are subsequently inadequate. They ignore the intricacies of working with organisms and parts from a range of risk groups, suggesting a systemic lag in the regulation. The fact that the legislation guiding the OGTR has not been updated since 2000, before the iGEM

competition began, further indicates that there is a fundamental gap between the advance of technology and the regulation, both legal and philosophical. Updates to this legislation, in recognition of the dynamic, integrated nature of genetic modification and the widespread uptake of synthetic biology amongst amateur scientists and others, is another essential component of inducing lasting change.

Conclusions

Ultimately, it is a clear that there are inherent problems within the philosophical paradigm of synthetic biology which allow potentially dangerous projects to proceed within iGEM. By considering synthetic biology as a means of generating synthetic pseudostrains, we can encourage amateur and professional users alike to conduct more thoughtful and thorough research into their chassis and donor organisms and their potential interactions. In the future, characterisation of these pseudostrains could provide a wealth of data indicating the risk of certain integrations. It is also evident that updates to the regulation in Australia are urgently needed to address the lag between technology and regulation. Increasing biosafety awareness in these ways will help to induce lasting changes in the young synthetic biology community, thereby ensuring that the iGEM competition remains a safe and positive reservoir of future synthetic biologists.

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